



Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **18E-22011**
Filename: **C:\Sirius_T3\18E-22011_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 12:19:28 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

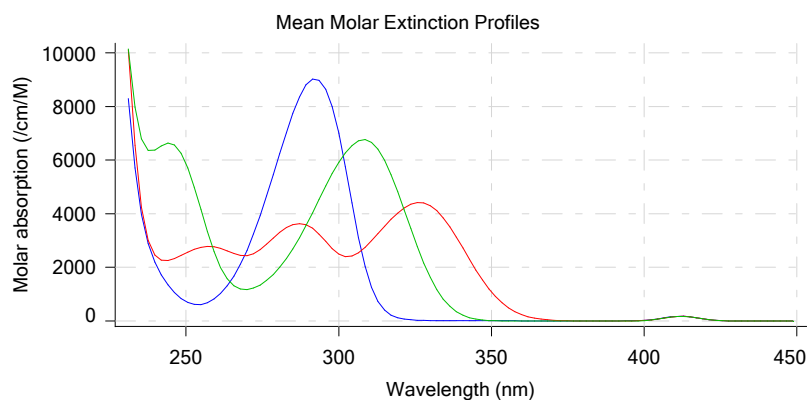
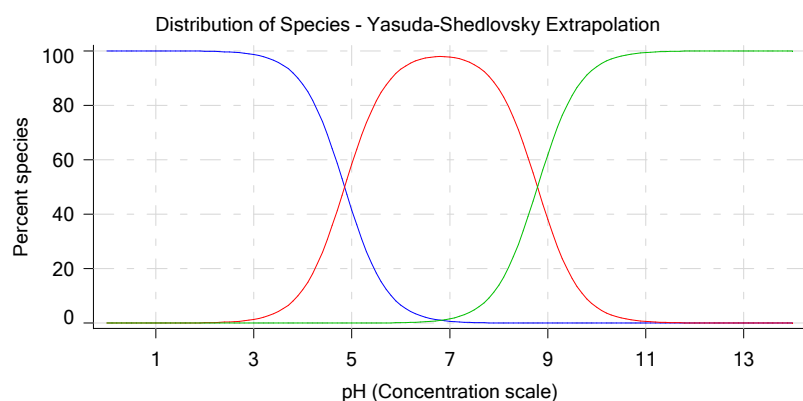
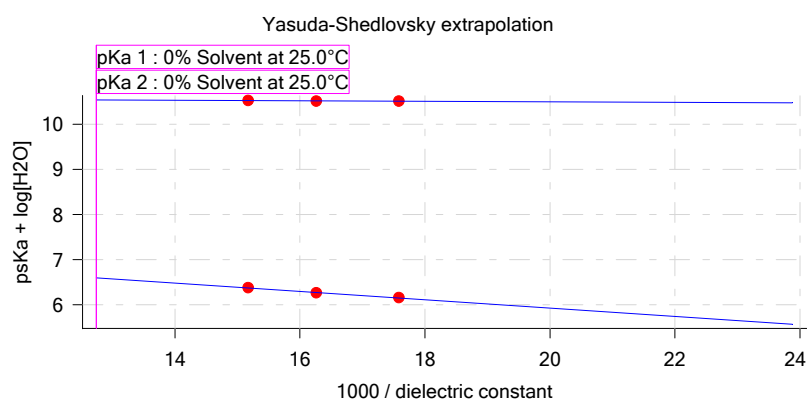
Yasuda-Shedlovsky result

Extrapolation type	pKa 0%	SD	Intercept	Slope	R ²	Ionic strength	Temperature
Yasuda-Shedlovsky	4.85	±0.02	7.76	-91.7353	0.9956	0.167 M	25.0°C
Yasuda-Shedlovsky	8.79	±0.01	10.61	-5.5912	0.8565	0.167 M	25.0°C

Component assay results

Titration	Methanol weight%	Direction	Result type	Dielectric constant	[H2O]	Ionic strength	Temperature	psKa 1	psKa 2
18E-22011 Points 4 to 43	48.92 %	Up	UV-metric pKa	56.9	25.0 M	0.158 M	25.0°C	✓ 4.76	✓ 9.11
18E-22011 Points 45 to 88	38.89 %	Up	UV-metric pKa	61.5	30.6 M	0.168 M	25.0°C	✓ 4.78	✓ 9.02
18E-22011 Points 90 to 135	29.14 %	Up	UV-metric pKa	65.9	36.4 M	0.175 M	25.0°C	✓ 4.82	✓ 8.96

Graphs



UV-metric psKa_0417936-0002 Titration 1 of 3 18E-22011 Points 4 to 43

Results

pKa 1 **4.76**
pKa 2 **9.11**
RMSD **0.037 0.030 0.024**
Chi squared **0.1587**
PCA calculated number of pKas **4**
Average ionic strength **0.158 M**
Average temperature **25.0°C**
Analyte concentration range **60.6 µM to 56.8 µM**
Methanol weight % **48.9 %**
Dielectric constant **56.9**
Water concentration **25.0 M**

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Results (continued)

Number of pKas source **Predicted**
 Wavelength clipping **230.0 nm to 450.0 nm**
 pH clipping **1.455 to 12.545**

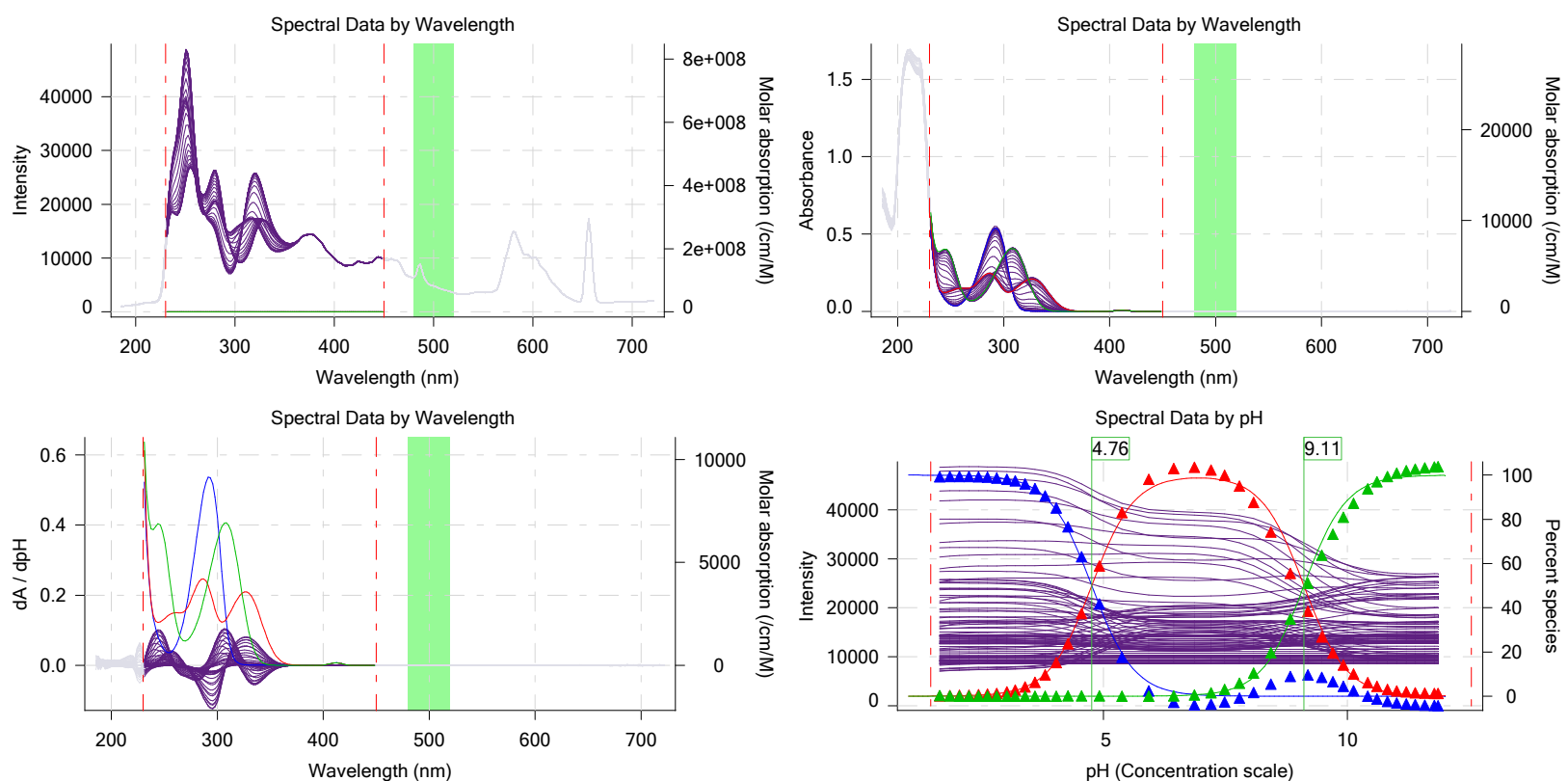
Warnings and errors

Errors None
 Warnings PCA calculation disagrees with predicted number of pKas

Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Assay Medium				
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			

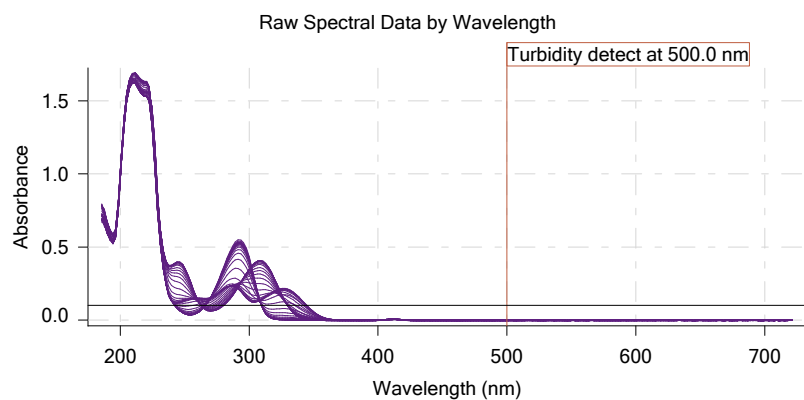
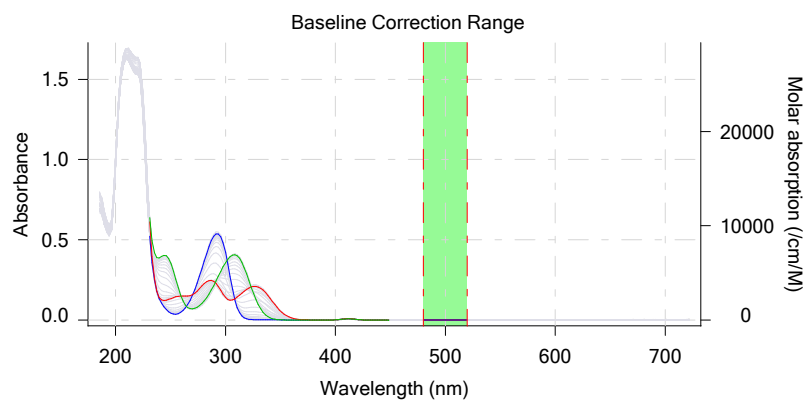
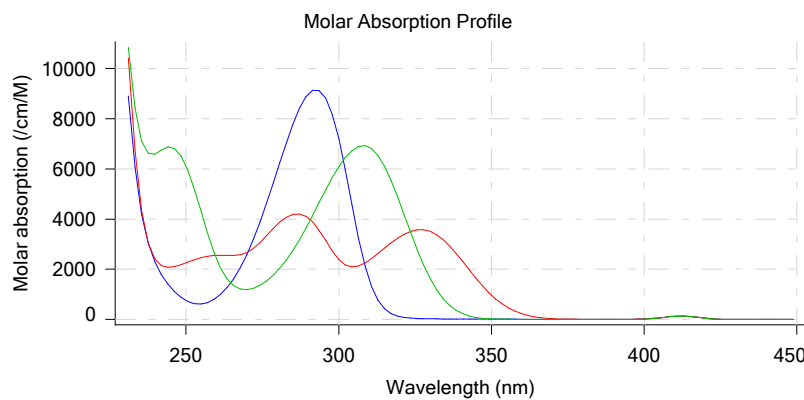
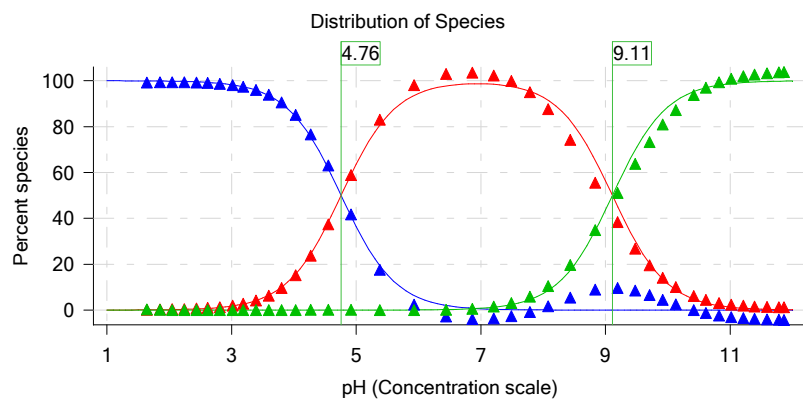
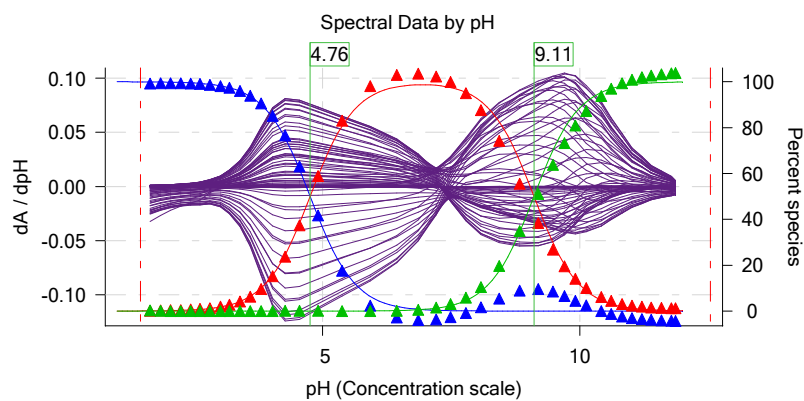
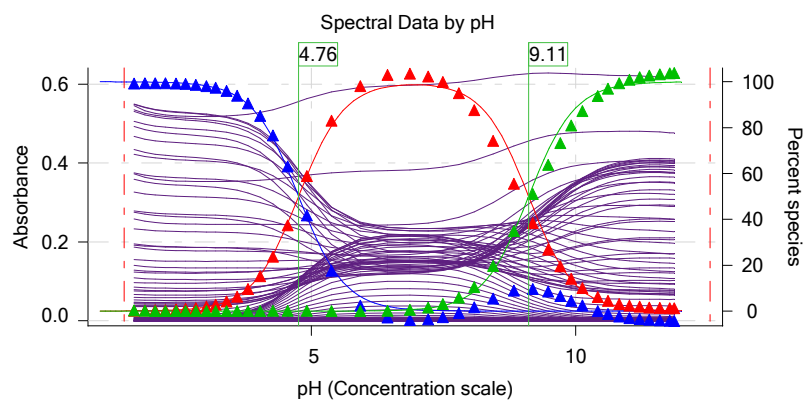
Graphs



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Experiment start time: **5/22/2018 12:19:28 PM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Graphs (continued)



UV-metric psKa_0417936-0002 Titration 2 of 3 18E-22011 Points 45 to 88

Results

pKa 1 **4.78**
 pKa 2 **9.02**
 RMSD **0.045 0.033 0.031**
 Chi squared **0.2321**
 PCA calculated number of pKas **3**
 Average ionic strength **0.168 M**
 Average temperature **25.0°C**
 Analyte concentration range **49.2 µM to 46.1 µM**
 Methanol weight % **38.9 %**
 Dielectric constant **61.5**
 Water concentration **30.6 M**

Sample name: **Pyridoxine HCl**
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 Analyst: **Dorothy Levorse**
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Results (continued)

Number of pKas source **Predicted**
 Wavelength clipping **230.0 nm to 450.0 nm**
 pH clipping **1.471 to 12.547**

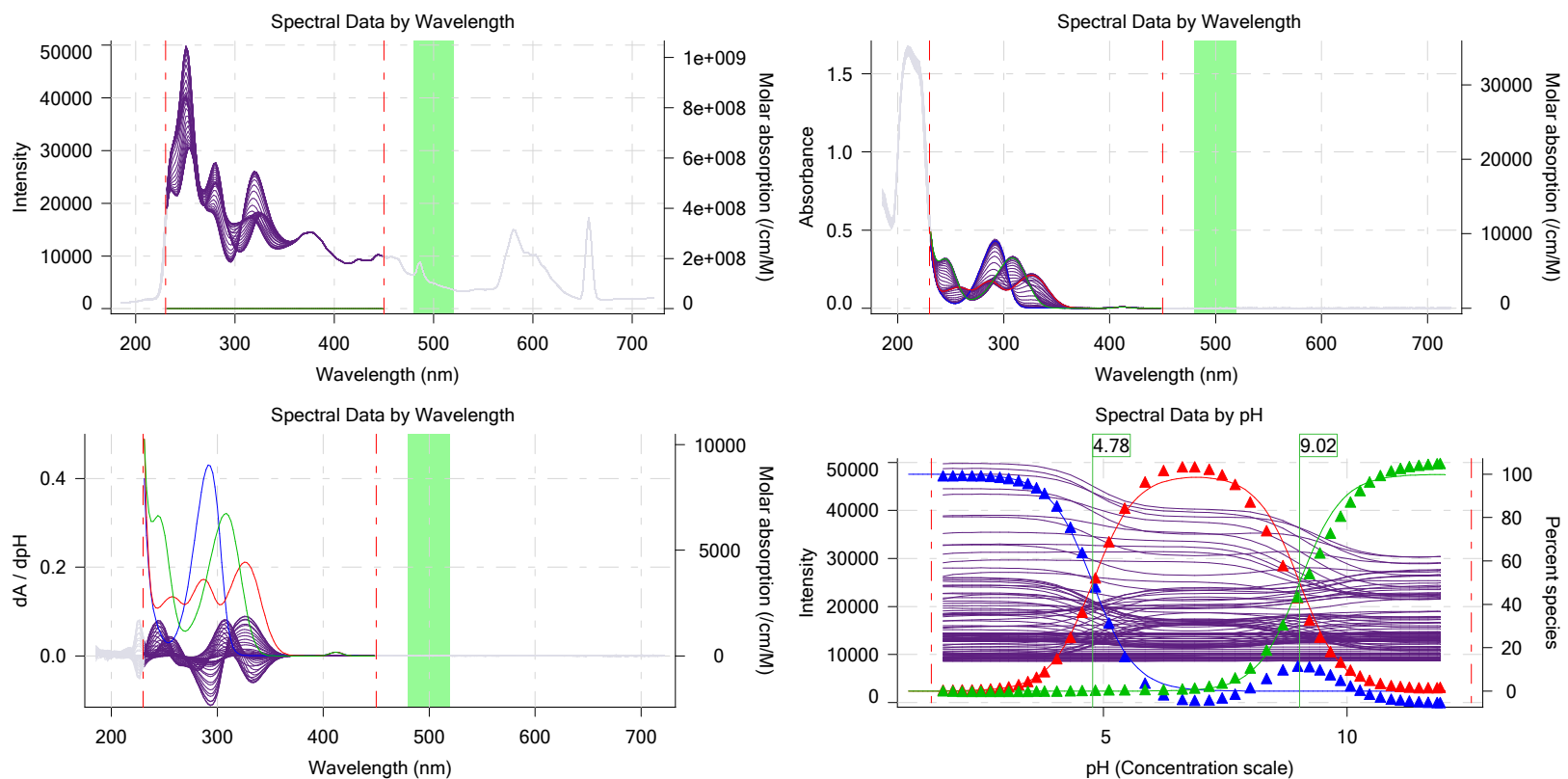
Warnings and errors

Errors **None**
 Warnings **PCA calculation disagrees with predicted number of pKas**

Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Assay Medium				
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			

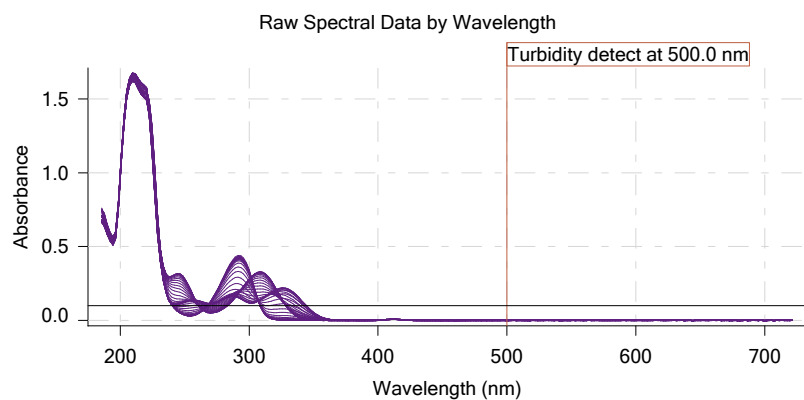
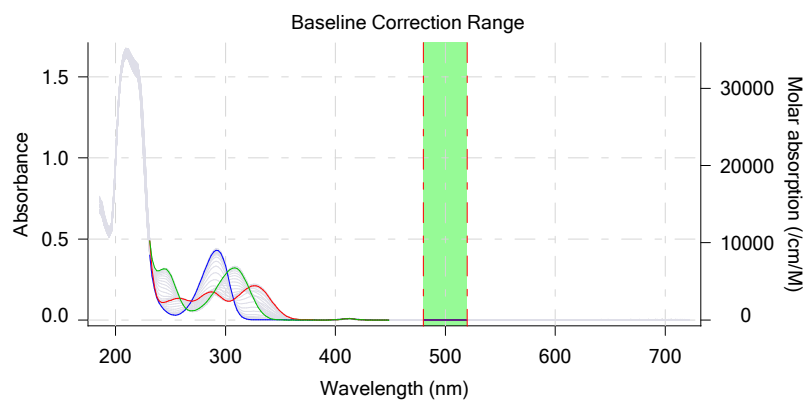
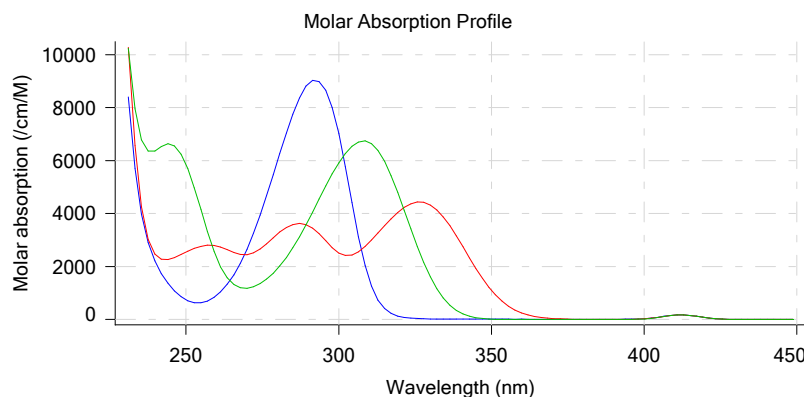
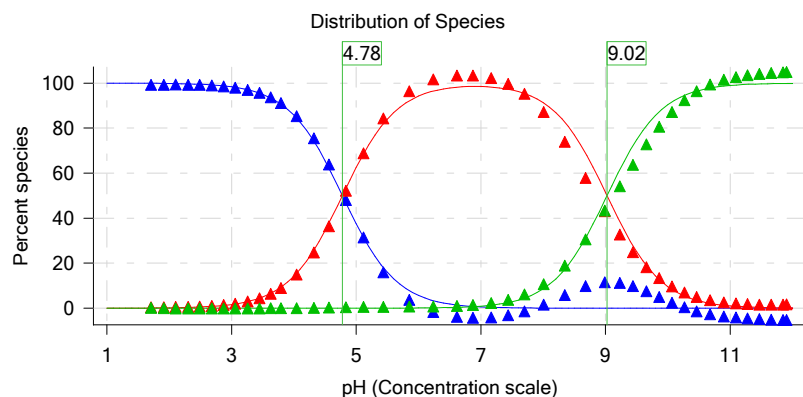
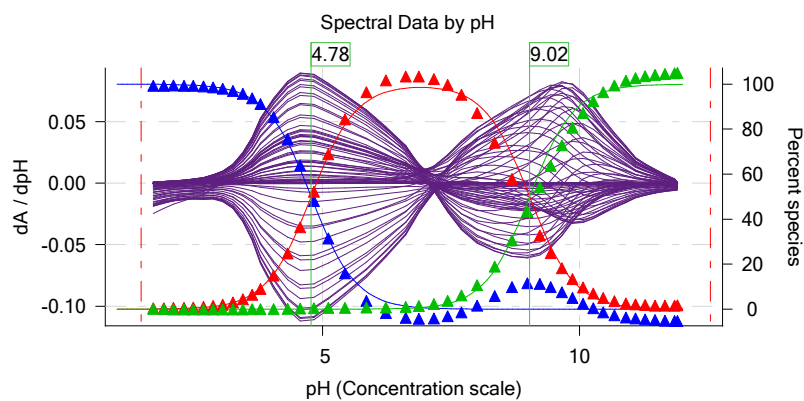
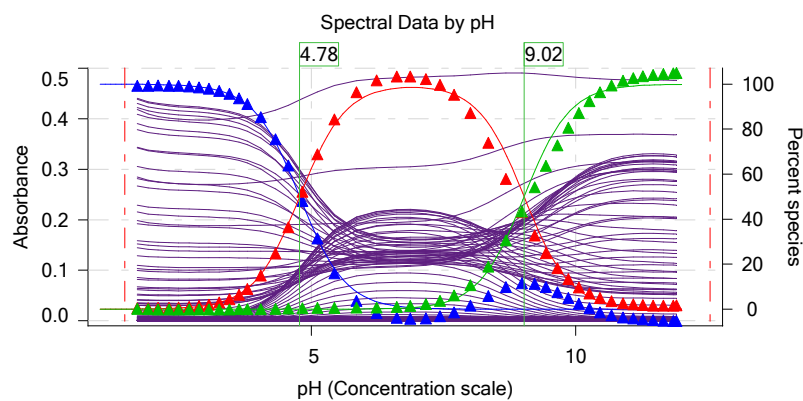
Graphs



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Experiment start time: **5/22/2018 12:19:28 PM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Graphs (continued)



UV-metric psKa_0417936-0002 Titration 3 of 3 18E-22011 Points 90 to 135

Results

pKa 1 **4.82**
 pKa 2 **8.96**
 RMSD **0.046 0.031 0.036**
 Chi squared **0.2797**
 PCA calculated number of pKas **3**
 Average ionic strength **0.175 M**
 Average temperature **25.0°C**
 Analyte concentration range **37.6 µM to 35.3 µM**
 Methanol weight % **29.1 %**
 Dielectric constant **65.9**
 Water concentration **36.4 M**

Sample name: **Pyridoxine HCl**
 Assay name: **UV-metric psKa**
 Assay ID: **18E-22011**
 Filename: **C:\Sirius_T3\18E-22011_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 12:19:28 PM**
 Analyst: **Dorothy Levorse**
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Results (continued)

Number of pKas source **Predicted**
 Wavelength clipping **230.0 nm to 450.0 nm**
 pH clipping **1.487 to 12.546**

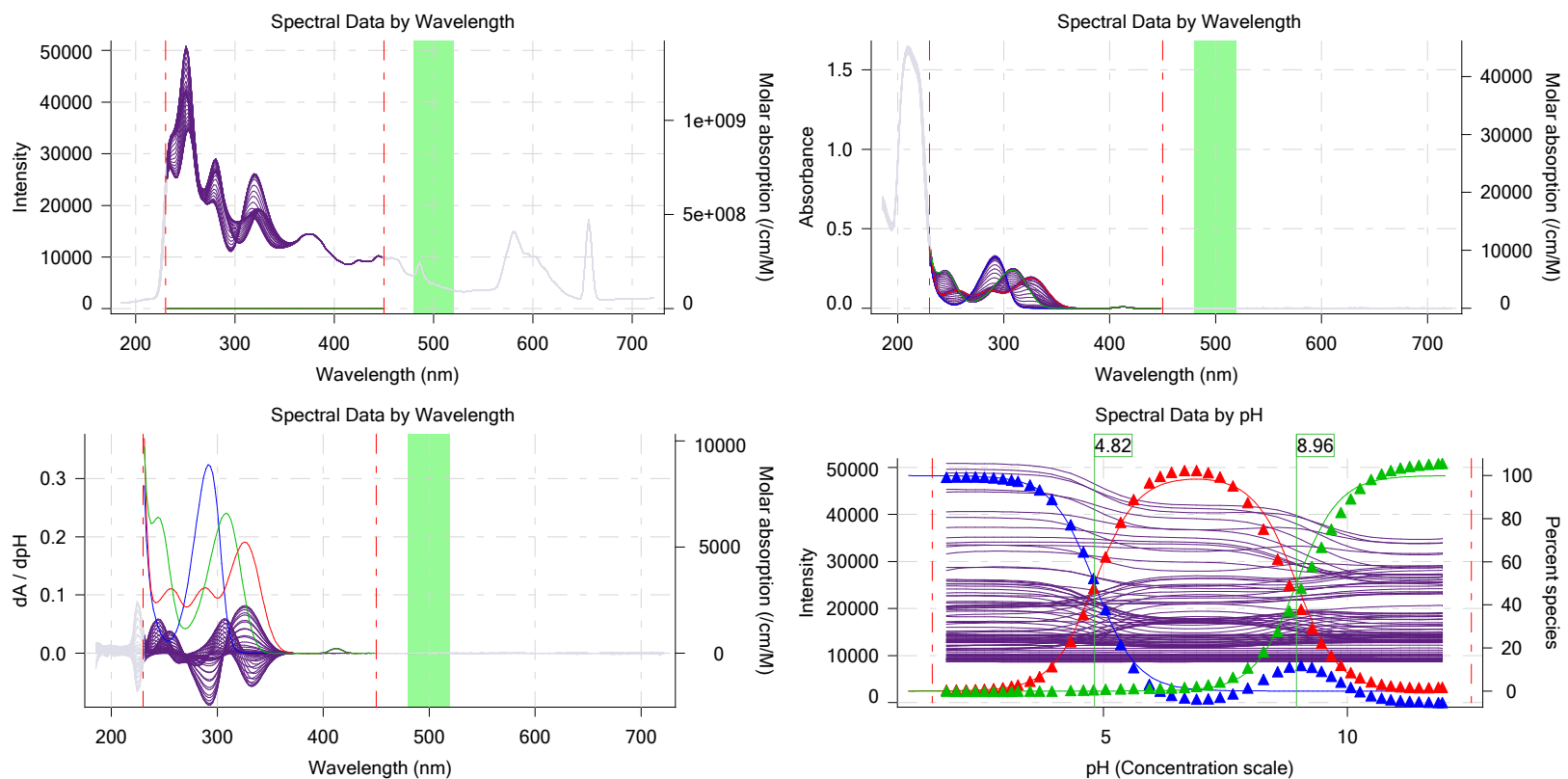
Warnings and errors

Errors None
 Warnings PCA calculation disagrees with predicted number of pKas

Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Assay Medium				
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			

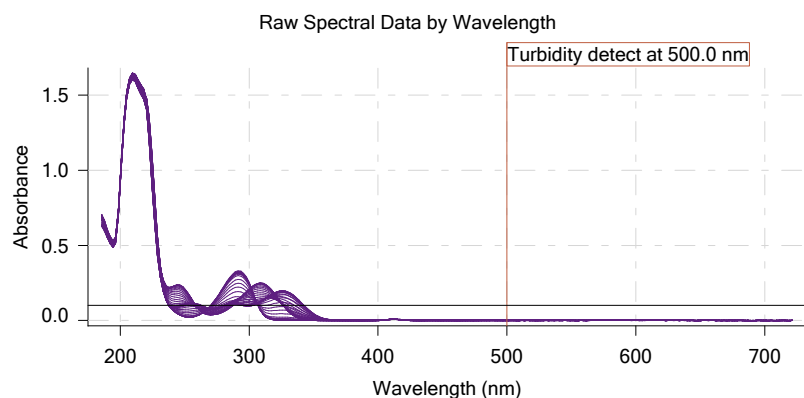
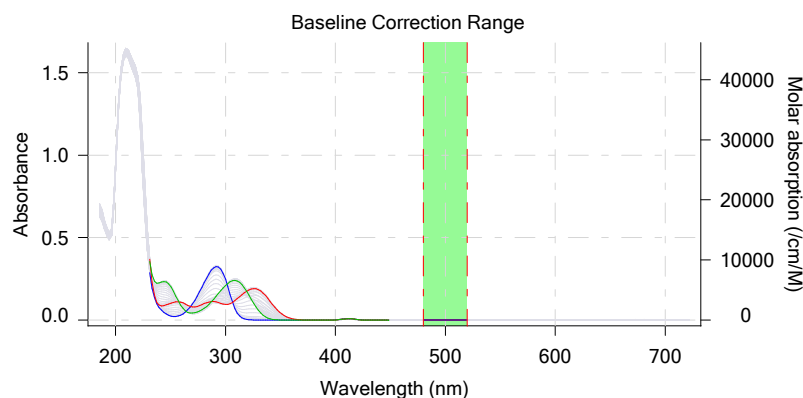
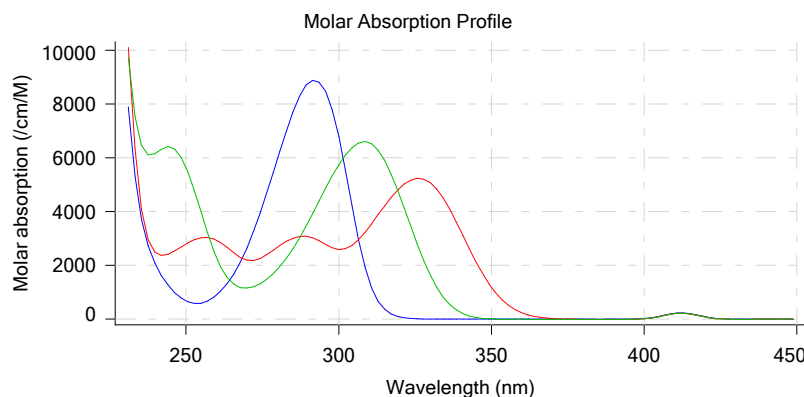
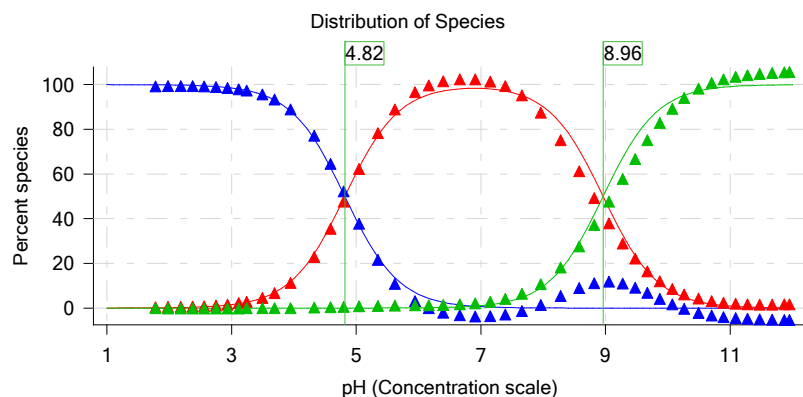
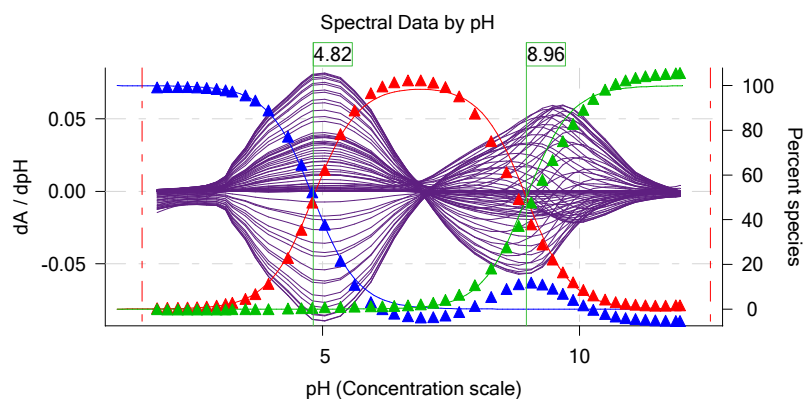
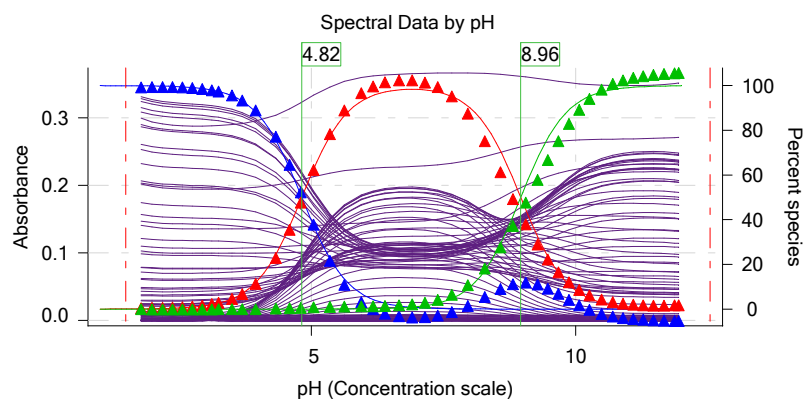
Graphs



Sample name: **Pyridoxine HCl**
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 Assay ID: **18E-22011**
 Filename: **C:\Sirius_T3\18E-22011_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 12:19:28 PM**
 Analyst: **Dorothy Levorse**
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Graphs (continued)



Assay Model

Settings

Settings	Value	Date/Time changed	Imported from
Sample name	Pyridoxine HCl	5/22/2018 9:07:27 AM	User entered value
Sample by	Volume		Default value
Sample volume	0.0020 mL	5/22/2018 9:07:27 AM	User entered value
Solvent	DMSO		Default value
Sample concentration	0.048630 M	5/22/2018 9:07:27 AM	User entered value
Solubility	Unknown		Default value
Molecular weight	205.64	5/22/2018 9:07:35 AM	User entered value
Individual pKa ionic environments	No		Default value
Number of pKas	2	5/22/2018 9:07:27 AM	User entered value
Sample is a	Ampholyte	5/22/2018 9:07:27 AM	User entered value
pKa 1	4.90	5/22/2018 9:07:27 AM	User entered value
Type	Base	5/22/2018 9:07:27 AM	User entered value
pKa 2	8.80	5/22/2018 9:07:27 AM	User entered value
Type	Acid	5/22/2018 9:07:27 AM	User entered value

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Assay Model (continued)

Settings	Value	Date/Time changed	Imported from
logp (XH2 +)	-10.00		Default value
logP (neutral XH)	-10.00	5/22/2018 9:07:27 AM	User entered value
logP (X -)	-10.00		Default value
Stoichiometry	1.00000		Default value
Aprotic counterion name	Chloride		From standards.xml file
Stoichiometry	1.00		From standards.xml file
Charge per counterion	-1		From standards.xml file

Events

Time	Event	Water	Acid	Base	Methanol	Buffer	pH	dpH/dt	pH R-square
3:47.3	Dark spectrum								
3:48.9	Reference spectrum								
4:16.5	Volume reset due to vial change								
5:00.7	Initial pH = 7.87								
6:10.4	Data point 4	0.34995 mL	0.07726 mL	0.00000 mL	1.15005 mL	0.02500 mL	1.955	-0.00772	0.61965
6:39.5	Data point 5	0.34995 mL	0.07726 mL	0.02813 mL	1.15005 mL	0.02500 mL	2.156	0.01115	0.56590
6:56.5	Data point 6	0.34995 mL	0.07726 mL	0.04501 mL	1.15005 mL	0.02500 mL	2.347	0.01384	0.57080
7:13.4	Data point 7	0.34995 mL	0.07726 mL	0.05588 mL	1.15005 mL	0.02500 mL	2.538	0.03397	0.85354
7:30.4	Data point 8	0.34995 mL	0.07726 mL	0.06284 mL	1.15005 mL	0.02500 mL	2.730	0.00492	0.31739
7:47.2	Data point 9	0.34995 mL	0.07726 mL	0.06733 mL	1.15005 mL	0.02500 mL	2.909	0.01020	0.66043
8:04.0	Data point 10	0.34995 mL	0.07726 mL	0.07030 mL	1.15005 mL	0.02500 mL	3.100	0.00803	0.74619
8:20.8	Data point 11	0.34995 mL	0.07726 mL	0.07220 mL	1.15005 mL	0.02500 mL	3.302	0.01901	0.96883
8:37.4	Data point 12	0.34995 mL	0.07726 mL	0.07338 mL	1.15005 mL	0.02500 mL	3.472	0.01793	0.88521
9:09.6	Data point 13	0.34995 mL	0.07726 mL	0.07441 mL	1.15005 mL	0.02500 mL	3.675	0.02585	0.91199
9:41.6	Data point 14	0.34995 mL	0.07726 mL	0.07505 mL	1.15005 mL	0.02500 mL	3.875	0.03952	0.97762
10:13.5	Data point 15	0.34995 mL	0.07726 mL	0.07549 mL	1.15005 mL	0.02500 mL	4.079	0.06259	0.96397
10:45.4	Data point 16	0.34995 mL	0.07726 mL	0.07603 mL	1.15005 mL	0.02500 mL	4.303	0.09345	0.99322
11:12.3	Data point 17	0.34995 mL	0.07726 mL	0.07627 mL	1.15005 mL	0.02500 mL	4.542	0.09632	0.98024
11:45.3	Data point 18	0.34995 mL	0.07726 mL	0.07653 mL	1.15005 mL	0.02500 mL	4.821	0.09636	0.97545
12:30.6	Data point 19	0.34995 mL	0.07726 mL	0.07667 mL	1.15005 mL	0.02500 mL	5.179	0.10014	0.99170
13:24.5	Data point 20	0.34995 mL	0.07726 mL	0.07679 mL	1.15005 mL	0.02500 mL	5.640	0.09945	0.99235
14:25.9	Data point 21	0.34995 mL	0.07726 mL	0.07688 mL	1.15005 mL	0.02500 mL	6.180	0.09930	0.99643
15:31.4	Data point 22	0.34995 mL	0.07726 mL	0.07698 mL	1.15005 mL	0.02500 mL	6.688	0.09832	0.99128
16:31.4	Data point 23	0.34995 mL	0.07726 mL	0.07709 mL	1.15005 mL	0.02500 mL	7.105	0.10060	0.99438
17:26.5	Data point 24	0.34995 mL	0.07726 mL	0.07723 mL	1.15005 mL	0.02500 mL	7.449	0.09811	0.98753
18:12.5	Data point 25	0.34995 mL	0.07726 mL	0.07738 mL	1.15005 mL	0.02500 mL	7.724	0.09986	0.98919
18:56.5	Data point 26	0.34995 mL	0.07726 mL	0.07754 mL	1.15005 mL	0.02500 mL	8.022	0.09930	0.98558
19:40.9	Data point 27	0.34995 mL	0.07726 mL	0.07768 mL	1.15005 mL	0.02500 mL	8.309	0.10075	0.99207
20:26.5	Data point 28	0.34995 mL	0.07726 mL	0.07782 mL	1.15005 mL	0.02500 mL	8.661	0.09753	0.98421
21:15.0	Data point 29	0.34995 mL	0.07726 mL	0.07796 mL	1.15005 mL	0.02500 mL	9.054	0.09457	0.97636
21:57.5	Data point 30	0.34995 mL	0.07726 mL	0.07810 mL	1.15005 mL	0.02500 mL	9.409	0.09700	0.98144
22:37.4	Data point 31	0.34995 mL	0.07726 mL	0.07825 mL	1.15005 mL	0.02500 mL	9.692	0.09580	0.98449
23:12.3	Data point 32	0.34995 mL	0.07726 mL	0.07839 mL	1.15005 mL	0.02500 mL	9.918	0.09682	0.98787
23:36.1	Data point 33	0.34995 mL	0.07726 mL	0.07855 mL	1.15005 mL	0.02500 mL	10.127	0.09847	0.96215
24:03.1	Data point 34	0.34995 mL	0.07726 mL	0.07879 mL	1.15005 mL	0.02500 mL	10.335	0.04977	0.96309
24:19.8	Data point 35	0.34995 mL	0.07726 mL	0.07912 mL	1.15005 mL	0.02500 mL	10.622	0.02210	0.94983
24:46.8	Data point 36	0.34995 mL	0.07726 mL	0.07966 mL	1.15005 mL	0.02500 mL	10.813	0.01386	0.91643
25:03.4	Data point 37	0.34995 mL	0.07726 mL	0.08062 mL	1.15005 mL	0.02500 mL	11.022	0.01074	0.92675
25:20.1	Data point 38	0.34995 mL	0.07726 mL	0.08215 mL	1.15005 mL	0.02500 mL	11.212	0.00860	0.60600
25:36.8	Data point 39	0.34995 mL	0.07726 mL	0.08452 mL	1.15005 mL	0.02500 mL	11.403	0.00392	0.54350
25:53.7	Data point 40	0.34995 mL	0.07726 mL	0.08822 mL	1.15005 mL	0.02500 mL	11.584	0.00233	0.25391
26:10.4	Data point 41	0.34995 mL	0.07726 mL	0.09386 mL	1.15005 mL	0.02500 mL	11.779	0.00676	0.80315
26:27.3	Data point 42	0.34995 mL	0.07726 mL	0.10282 mL	1.15005 mL	0.02500 mL	11.967	0.00481	0.56597
26:44.2	Data point 43	0.34995 mL	0.07726 mL	0.10786 mL	1.15005 mL	0.02500 mL	12.045	0.00461	0.59794
28:26.5	Reference spectrum								
29:31.0	Data point 45	0.50000 mL	0.19334 mL	0.10788 mL	1.15005 mL	0.02500 mL	1.971	-0.10001	0.97843

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 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Events (continued)

Time	Event	Water	Acid	Base	Methanol	Buffer	pH	dpH/dt	pH R-squared	pH SD
30:00.9	Data point 46	0.50000 mL	0.19334 mL	0.14059 mL	1.15005 mL	0.02500 mL	2.168	0.00102	0.05316	0.00
30:17.9	Data point 47	0.50000 mL	0.19334 mL	0.15882 mL	1.15005 mL	0.02500 mL	2.352	0.01120	0.81812	0.00
30:35.0	Data point 48	0.50000 mL	0.19334 mL	0.17063 mL	1.15005 mL	0.02500 mL	2.550	0.00693	0.30426	0.00
30:51.9	Data point 49	0.50000 mL	0.19334 mL	0.17801 mL	1.15005 mL	0.02500 mL	2.729	0.00261	0.04275	0.00
31:08.8	Data point 50	0.50000 mL	0.19334 mL	0.18290 mL	1.15005 mL	0.02500 mL	2.926	0.01737	0.83620	0.00
31:25.5	Data point 51	0.50000 mL	0.19334 mL	0.18596 mL	1.15005 mL	0.02500 mL	3.110	0.01260	0.81884	0.00
31:42.3	Data point 52	0.50000 mL	0.19334 mL	0.18798 mL	1.15005 mL	0.02500 mL	3.296	0.00889	0.80694	0.00
31:59.0	Data point 53	0.50000 mL	0.19334 mL	0.18930 mL	1.15005 mL	0.02500 mL	3.491	0.01995	0.97079	0.00
32:15.7	Data point 54	0.50000 mL	0.19334 mL	0.19012 mL	1.15005 mL	0.02500 mL	3.679	0.01497	0.86623	0.00
32:32.4	Data point 55	0.50000 mL	0.19334 mL	0.19066 mL	1.15005 mL	0.02500 mL	3.859	0.03662	0.98535	0.00
32:49.0	Data point 56	0.50000 mL	0.19334 mL	0.19102 mL	1.15005 mL	0.02500 mL	4.020	0.03589	0.83279	0.00
33:10.8	Data point 57	0.50000 mL	0.19334 mL	0.19139 mL	1.15005 mL	0.02500 mL	4.271	0.06012	0.99357	0.00
33:32.7	Data point 58	0.50000 mL	0.19334 mL	0.19165 mL	1.15005 mL	0.02500 mL	4.543	0.08457	0.98921	0.00
33:54.5	Data point 59	0.50000 mL	0.19334 mL	0.19182 mL	1.15005 mL	0.02500 mL	4.783	0.09749	0.97508	0.00
34:31.9	Data point 60	0.50000 mL	0.19334 mL	0.19196 mL	1.15005 mL	0.02500 mL	5.051	0.09819	0.99229	0.00
35:08.3	Data point 61	0.50000 mL	0.19334 mL	0.19207 mL	1.15005 mL	0.02500 mL	5.330	0.09758	0.97956	0.00
35:50.2	Data point 62	0.50000 mL	0.19334 mL	0.19217 mL	1.15005 mL	0.02500 mL	5.649	0.09843	0.98652	0.00
36:35.6	Data point 63	0.50000 mL	0.19334 mL	0.19226 mL	1.15005 mL	0.02500 mL	6.059	0.10020	0.98033	0.00
37:22.4	Data point 64	0.50000 mL	0.19334 mL	0.19236 mL	1.15005 mL	0.02500 mL	6.440	0.09816	0.98715	0.00
38:15.9	Data point 65	0.50000 mL	0.19334 mL	0.19247 mL	1.15005 mL	0.02500 mL	6.818	0.09837	0.99063	0.00
38:58.3	Data point 66	0.50000 mL	0.19334 mL	0.19259 mL	1.15005 mL	0.02500 mL	7.071	0.10035	0.98967	0.00
39:39.3	Data point 67	0.50000 mL	0.19334 mL	0.19273 mL	1.15005 mL	0.02500 mL	7.362	0.09739	0.97312	0.00
40:18.2	Data point 68	0.50000 mL	0.19334 mL	0.19287 mL	1.15005 mL	0.02500 mL	7.628	0.09805	0.97970	0.00
40:57.7	Data point 69	0.50000 mL	0.19334 mL	0.19301 mL	1.15005 mL	0.02500 mL	7.890	0.09989	0.98284	0.00
41:37.8	Data point 70	0.50000 mL	0.19334 mL	0.19318 mL	1.15005 mL	0.02500 mL	8.195	0.09900	0.97814	0.00
42:20.8	Data point 71	0.50000 mL	0.19334 mL	0.19334 mL	1.15005 mL	0.02500 mL	8.530	0.09664	0.98007	0.00
43:05.3	Data point 72	0.50000 mL	0.19334 mL	0.19348 mL	1.15005 mL	0.02500 mL	8.862	0.09418	0.98569	0.00
43:41.7	Data point 73	0.50000 mL	0.19334 mL	0.19365 mL	1.15005 mL	0.02500 mL	9.163	0.09925	0.98365	0.00
44:22.0	Data point 74	0.50000 mL	0.19334 mL	0.19384 mL	1.15005 mL	0.02500 mL	9.403	0.09820	0.96242	0.00
44:57.0	Data point 75	0.50000 mL	0.19334 mL	0.19419 mL	1.15005 mL	0.02500 mL	9.614	0.09205	0.96674	0.00
45:30.6	Data point 76	0.50000 mL	0.19334 mL	0.19447 mL	1.15005 mL	0.02500 mL	9.827	0.06575	0.97089	0.00
45:52.3	Data point 77	0.50000 mL	0.19334 mL	0.19471 mL	1.15005 mL	0.02500 mL	10.031	0.04335	0.97625	0.00
46:24.4	Data point 78	0.50000 mL	0.19334 mL	0.19506 mL	1.15005 mL	0.02500 mL	10.231	0.02376	0.93736	0.00
46:56.6	Data point 79	0.50000 mL	0.19334 mL	0.19553 mL	1.15005 mL	0.02500 mL	10.429	0.01865	0.93667	0.00
47:28.7	Data point 80	0.50000 mL	0.19334 mL	0.19617 mL	1.15005 mL	0.02500 mL	10.624	0.00749	0.83005	0.00
47:45.4	Data point 81	0.50000 mL	0.19334 mL	0.19704 mL	1.15005 mL	0.02500 mL	10.839	0.00505	0.79553	0.00
48:02.1	Data point 82	0.50000 mL	0.19334 mL	0.19847 mL	1.15005 mL	0.02500 mL	11.058	0.00179	0.27424	0.00
48:18.8	Data point 83	0.50000 mL	0.19334 mL	0.20082 mL	1.15005 mL	0.02500 mL	11.246	0.00719	0.81073	0.00
48:35.6	Data point 84	0.50000 mL	0.19334 mL	0.20444 mL	1.15005 mL	0.02500 mL	11.429	0.00340	0.52423	0.00
48:52.3	Data point 85	0.50000 mL	0.19334 mL	0.20997 mL	1.15005 mL	0.02500 mL	11.619	0.00710	0.73496	0.00
49:09.2	Data point 86	0.50000 mL	0.19334 mL	0.21860 mL	1.15005 mL	0.02500 mL	11.805	0.00367	0.39432	0.00
49:26.1	Data point 87	0.50000 mL	0.19334 mL	0.23210 mL	1.15005 mL	0.02500 mL	11.988	0.00362	0.43612	0.00
49:43.0	Data point 88	0.50000 mL	0.19334 mL	0.23758 mL	1.15005 mL	0.02500 mL	12.047	0.00577	0.75458	0.00
51:50.1	Reference spectrum									
52:58.3	Data point 90	0.83996 mL	0.33483 mL	0.23761 mL	1.15005 mL	0.02500 mL	1.987	-0.09876	0.98599	0.00
53:42.9	Data point 91	0.83996 mL	0.33483 mL	0.27587 mL	1.15005 mL	0.02500 mL	2.181	-0.00579	0.52710	0.00
54:10.4	Data point 92	0.83996 mL	0.33483 mL	0.29694 mL	1.15005 mL	0.02500 mL	2.379	0.00234	0.11016	0.00
54:27.4	Data point 93	0.83996 mL	0.33483 mL	0.30974 mL	1.15005 mL	0.02500 mL	2.566	-0.01701	0.86551	0.00
54:44.2	Data point 94	0.83996 mL	0.33483 mL	0.31809 mL	1.15005 mL	0.02500 mL	2.748	0.00081	0.00429	0.00
55:01.1	Data point 95	0.83996 mL	0.33483 mL	0.32349 mL	1.15005 mL	0.02500 mL	2.936	-0.00425	0.29064	0.00
55:17.9	Data point 96	0.83996 mL	0.33483 mL	0.32700 mL	1.15005 mL	0.02500 mL	3.121	-0.01662	0.83449	0.00
55:34.6	Data point 97	0.83996 mL	0.33483 mL	0.32930 mL	1.15005 mL	0.02500 mL	3.298	-0.00866	0.53756	0.00
55:51.3	Data point 98	0.83996 mL	0.33483 mL	0.33081 mL	1.15005 mL	0.02500 mL	3.426	-0.00171	0.08882	0.00
56:23.4	Data point 99	0.83996 mL	0.33483 mL	0.33224 mL	1.15005 mL	0.02500 mL	3.675	0.00595	0.67554	0.00
56:50.3	Data point 100	0.83996 mL	0.33483 mL	0.33281 mL	1.15005 mL	0.02500 mL	3.867	0.01093	0.89224	0.00
57:07.0	Data point 101	0.83996 mL	0.33483 mL	0.33321 mL	1.15005 mL	0.02500 mL	4.120	-0.00252	0.05659	0.00
57:28.8	Data point 102	0.83996 mL	0.33483 mL	0.33363 mL	1.15005 mL	0.02500 mL	4.497	-0.00454	0.08769	0.00

Sample name: **Pyridoxine HCl**
 Assay name: **UV-metric psKa**
 Assay ID: **18E-22011**
 Filename: **C:\Sirius_T3\18E-22011_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 12:19:28 PM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Events (continued)

Time	Event	Water	Acid	Base	Methanol	Buffer	pH	dpH/dt	pH R-squared	pH SD
57:50.5	Data point 103	0.83996 mL	0.33483 mL	0.33384 mL	1.15005 mL	0.02500 mL	4.759	0.03419	0.95872	0.0017
58:12.4	Data point 104	0.83996 mL	0.33483 mL	0.33398 mL	1.15005 mL	0.02500 mL	4.967	0.08527	0.98858	0.0042
58:39.3	Data point 105	0.83996 mL	0.33483 mL	0.33410 mL	1.15005 mL	0.02500 mL	5.210	0.09671	0.99101	0.0047
59:11.6	Data point 106	0.83996 mL	0.33483 mL	0.33422 mL	1.15005 mL	0.02500 mL	5.513	0.09710	0.97457	0.0048
59:40.9	Data point 107	0.83996 mL	0.33483 mL	0.33431 mL	1.15005 mL	0.02500 mL	5.782	0.09877	0.98203	0.0049
1:00:20.8	Data point 108	0.83996 mL	0.33483 mL	0.33441 mL	1.15005 mL	0.02500 mL	6.100	0.09969	0.98809	0.0049
1:00:54.1	Data point 109	0.83996 mL	0.33483 mL	0.33450 mL	1.15005 mL	0.02500 mL	6.321	0.08279	0.76909	0.0046
1:01:15.8	Data point 110	0.83996 mL	0.33483 mL	0.33459 mL	1.15005 mL	0.02500 mL	6.548	0.07039	0.65422	0.0043
1:01:37.6	Data point 111	0.83996 mL	0.33483 mL	0.33471 mL	1.15005 mL	0.02500 mL	6.812	0.04591	0.28713	0.0042
1:02:05.0	Data point 112	0.83996 mL	0.33483 mL	0.33485 mL	1.15005 mL	0.02500 mL	7.057	0.08431	0.86462	0.0044
1:02:32.4	Data point 113	0.83996 mL	0.33483 mL	0.33499 mL	1.15005 mL	0.02500 mL	7.298	0.08343	0.81431	0.0045
1:03:06.0	Data point 114	0.83996 mL	0.33483 mL	0.33514 mL	1.15005 mL	0.02500 mL	7.535	0.08886	0.92103	0.0045
1:03:39.8	Data point 115	0.83996 mL	0.33483 mL	0.33528 mL	1.15005 mL	0.02500 mL	7.802	0.09817	0.95470	0.0049
1:04:16.3	Data point 116	0.83996 mL	0.33483 mL	0.33542 mL	1.15005 mL	0.02500 mL	8.100	0.09469	0.95852	0.0047
1:04:54.4	Data point 117	0.83996 mL	0.33483 mL	0.33556 mL	1.15005 mL	0.02500 mL	8.419	0.09873	0.95441	0.0049
1:05:33.4	Data point 118	0.83996 mL	0.33483 mL	0.33570 mL	1.15005 mL	0.02500 mL	8.710	0.09479	0.96226	0.0047
1:06:06.4	Data point 119	0.83996 mL	0.33483 mL	0.33584 mL	1.15005 mL	0.02500 mL	8.949	0.09165	0.93096	0.0046
1:06:41.5	Data point 120	0.83996 mL	0.33483 mL	0.33601 mL	1.15005 mL	0.02500 mL	9.185	0.08075	0.94036	0.0041
1:07:13.4	Data point 121	0.83996 mL	0.33483 mL	0.33620 mL	1.15005 mL	0.02500 mL	9.406	0.05660	0.96063	0.0028
1:07:35.2	Data point 122	0.83996 mL	0.33483 mL	0.33638 mL	1.15005 mL	0.02500 mL	9.601	0.02231	0.95508	0.0011
1:08:07.2	Data point 123	0.83996 mL	0.33483 mL	0.33666 mL	1.15005 mL	0.02500 mL	9.797	0.01732	0.87678	0.0009
1:08:39.3	Data point 124	0.83996 mL	0.33483 mL	0.33704 mL	1.15005 mL	0.02500 mL	9.994	0.02135	0.94744	0.0010
1:09:11.3	Data point 125	0.83996 mL	0.33483 mL	0.33753 mL	1.15005 mL	0.02500 mL	10.191	0.01065	0.91235	0.0005
1:09:43.6	Data point 126	0.83996 mL	0.33483 mL	0.33826 mL	1.15005 mL	0.02500 mL	10.384	0.00562	0.66301	0.0003
1:10:05.5	Data point 127	0.83996 mL	0.33483 mL	0.33946 mL	1.15005 mL	0.02500 mL	10.611	-0.01477	0.84873	0.0007
1:10:37.7	Data point 128	0.83996 mL	0.33483 mL	0.34099 mL	1.15005 mL	0.02500 mL	10.819	-0.00454	0.61071	0.0002
1:11:04.7	Data point 129	0.83996 mL	0.33483 mL	0.34271 mL	1.15005 mL	0.02500 mL	11.009	-0.00196	0.21283	0.0002
1:11:31.8	Data point 130	0.83996 mL	0.33483 mL	0.34591 mL	1.15005 mL	0.02500 mL	11.201	-0.00222	0.22145	0.0002
1:11:48.6	Data point 131	0.83996 mL	0.33483 mL	0.35089 mL	1.15005 mL	0.02500 mL	11.390	-0.01446	0.84982	0.0007
1:12:05.6	Data point 132	0.83996 mL	0.33483 mL	0.35866 mL	1.15005 mL	0.02500 mL	11.579	-0.01315	0.83717	0.0007
1:12:22.7	Data point 133	0.83996 mL	0.33483 mL	0.37081 mL	1.15005 mL	0.02500 mL	11.777	-0.00757	0.43491	0.0005
1:12:39.8	Data point 134	0.83996 mL	0.33483 mL	0.39031 mL	1.15005 mL	0.02500 mL	11.969	-0.01415	0.71005	0.0008
1:12:56.8	Data point 135	0.83996 mL	0.33483 mL	0.40122 mL	1.15005 mL	0.02500 mL	12.046	-0.00446	0.44815	0.0003
1:15:02.2	Assay volumes	1.08996 mL	0.50520 mL	0.40122 mL	1.15005 mL	0.02500 mL				

Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
General Settings				
Analyst name	Dorothy Levorse			
Separate reference vial	Yes			
Standard Experiment Settings				
Number of titrations	3			
Minimum pH	2.000			
Maximum pH	12.000			
pH step between points of	0.200			
Minimum titrant addition	0.00002 mL			
Maximum titrant addition	0.10000 mL			
Argon flow rate	100%			
Start titration using	Cautious pH adjust			
Advanced General Settings				
Detect turbidity using	Spectrometer			
Monitor at a wavelength of	500.0 nm			
Absorbance threshold of	0.100			
Collect turbidity sensor data	No			
Stir after titrant addition for	5 seconds			
For titrant addition, stir at	15%			
Titant Pre-Dose				



Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **18E-22011**
Filename: **C:\Sirius_T3\18E-22011_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 12:19:28 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Assay Settings (continued)

Setting	Value	Original Value	Date/Time changed	Imported from
Titration pre-dose	None			
Assay Medium				
Cosolvent in use	Yes			
Cosolvent type	Methanol			
Cosolvent volume	1.15 mL			
Cosolvent added	Automatic			
ISA water volume	0.35 mL			
Water added	Automatic			
After water addition, stir for	5 seconds			
At a speed of	15%			
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			
After medium addition, stir for	5 seconds			
Sample Sonication				
Sonicate	No			
Sample Dissolution				
Perform a dissolution stage	No			
Carbonate purge				
Perform a carbonate purge	No			
Temperature Control				
Wait for temperature	Yes			
Required start temperature	25.0°C			
Acceptable deviation	0.5°C			
Time to wait	60 seconds			
Stir speed of	15%			
Titration 1				
Titrate from	Low to high pH			
Adjust to start pH	Yes			
After pH adjust stir for	10 seconds			
Titration 2				
Titrate from	Low to high pH			
Additional cosolvent volume	0.00 mL			
Add additional water	0.15 mL			
Additional water added	Automatic			
After pH adjust stir for	10 seconds			
Titration 3				
Titrate from	Low to high pH			
Additional cosolvent volume	0.00 mL			
Add additional water	0.34 mL			
Additional water added	Automatic			
After pH adjust stir for	10 seconds			
Data Point Stability				
Stir during data point collection	Yes			
For point collection, stir at	15%			
Delay before data point collection	0 seconds			
Number of points to average	20 points			
Time interval between points	0.50 seconds			
Required maximum standard deviation	0.00500 dpH/dt			
Stability timeout after	60 seconds			
Experiment cleanup				
Adjust pH to cleanup	To start pH			
And then stir for	60 seconds			
For cleaning, stir at	20%			
Then add water volume	0.25 mL			
And then stir for	30 seconds			



Sample name: **Pyridoxine HCl** Experiment start time: **5/22/2018 12:19:28 PM**
Assay name: **UV-metric psKa** Analyst: **Dorothy Levorse**
Assay ID: **18E-22011** Instrument ID: **T311053**
Filename: **C:\Sirius_T3\18E-22011_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Calibration Settings

Setting	Value	Date/Time changed	Imported from
Four-Plus alpha	0.144	5/22/2018 12:19:28 PM	C:\Sirius_T3\18E-22009_Blank standardisation.t3r
Four-Plus S	0.9948	5/22/2018 12:19:28 PM	C:\Sirius_T3\18E-22009_Blank standardisation.t3r
Four-Plus jH	1.0	5/22/2018 12:19:28 PM	C:\Sirius_T3\18E-22009_Blank standardisation.t3r
Four-Plus jOH	-0.8	5/22/2018 12:19:28 PM	C:\Sirius_T3\18E-22009_Blank standardisation.t3r
Base concentration factor	1.012	5/22/2018 12:19:28 PM	C:\Sirius_T3\KOH18D10.t3r
Acid concentration factor	0.998	5/22/2018 12:19:28 PM	C:\Sirius_T3\18E-22009_Blank standardisation.t3r

Instrument Settings

Setting	Value	Batch Id	Install date
Instrument owner	Merck		
Instrument ID	T311053		
Instrument type	T3 Simulator		
Software version	1.1.3.0		
Dispenser module		T3DM1100253	3/31/2009 6:24:52 AM
Dispenser 0	Water		3/31/2009 6:25:05 AM
Syringe volume	2.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Water (0.15 M KCl)	2-6-18	5/15/2018 2:12:22 PM
Dispenser 2	Acid		3/31/2009 6:25:11 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Acid (0.5 M HCl)	3-22-18	5/15/2018 2:12:48 PM
Dispenser 1	Base		3/31/2009 6:25:21 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Base (0.5 M KOH)	3-22-18	5/15/2018 2:12:34 PM
Dispenser 5	Cosolvent		3/31/2009 6:26:24 AM
Syringe volume	2.5 mL		
Firmware version	1.2.1(r2)		
Distribution valve 5	Distribution Valve		3/31/2009 6:28:19 AM
Firmware version	1.1.3		
Port A	Methanol (80%, 0.15 M KCl)	2-8-18	5/15/2018 2:14:14 PM
Port B	Cyclohexane		4/10/2018 8:40:51 AM
Port C	MeCN (50%, 0.15 M KCl)	4-16-18	5/15/2018 2:14:20 PM
Dispenser 3	Buffer		8/3/2010 6:05:16 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Dodecane	1-31-2018	5/15/2018 2:12:54 PM
Dispenser 6	Octanol		10/22/2010 11:52:43 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Octanol	1-31-2018	4/9/2018 9:14:11 AM
Titrator		T3TM1100153	3/31/2009 6:24:17 AM
Horizontal axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Vertical axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Chassis I/O firmware version	1.11 AI1DI0DO4 Norgren I/O		
Probe I/O firmware version	1.1.1		
Electrode	T3 Electrode	T3E0769	8/15/2017 10:21:54 AM
E0 calibration	-10.62 mV		5/22/2018 12:19:52 PM
Filling solution	3M KCl	KCL095	5/21/2018 8:57:01 AM
Liquids			
Wash 1	50% IPA:50% Water		5/22/2018 8:38:15 AM
Wash 2	0.5% Triton X-100 in H2O		5/22/2018 8:38:18 AM
Buffer position 1	pH7 Wash		5/22/2018 8:38:22 AM
Buffer position 2	pH 7		5/22/2018 8:38:25 AM
Storage position			5/22/2018 8:38:32 AM
Wash water	3.5e+003 mL	5-15-18	5/15/2018 2:11:48 PM



Assay Settings

Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **18E-22011**
Filename: **C:\Sirius_T3\18E-22011_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 12:19:28 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Instrument Settings (continued)

Setting	Value	Batch Id	Install date
Waste	7e+003 mL		3/19/2018 10:48:12 AM
Temperature controller			8/5/2010 7:35:13 AM
Turbidity detector			3/31/2009 6:24:45 AM
Spectrometer		072390	11/23/2010 12:22:28 PM
Dip probe		11086	
Wavelength coefficient A0	185.563		
Wavelength coefficient A1	2.17439		
Wavelength coefficient A2	-0.000285622		
Total lamp lit time	897:26:49		11/23/2010 12:22:28 PM
Calibrated on	5/21/2018 2:44:22 PM		
Integration time	19		
Scans averaged	10		
Autoloader		T3AL1100237	11/10/2015 10:34:13 AM
Left-right axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Front-back axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Vertical axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Chassis I/O firmware version	1.11 AI1DI0DO4 Norgren I/O		
Configuration			
Alternate titration position	Titration position		
Alternate reference position	Reference position		
Maximum standard vial volume	3.50 mL		
Maximum alternate vial volume	25.00 mL		
Automatic action idle period	5 minute(s)		
Titrant tube volume	1.3 mL		
Syringe flush count	3.50		
Flowing wash pump volume	20.0 mL		
Flowing wash stir duration	5 s		
Flowing wash stir speed	30%		
Solvent wash stir duration	5 s		
Solvent wash stir speed	30%		
Surfactant wash stir duration	5 s		
Surfactant wash stir speed	30%		
E0 calibration minimum number of points	10		
E0 calibration maximum standard deviation	0.01500		
E0 calibration timeout period	60 s		
E0 calibration stir duration	5 s		
E0 calibration preparation stir speed	30%		
E0 calibration buffer wash stir duration	5 s		
E0 calibration buffer wash stir speed	30%		
E0 calibration reading stir speed	0%		
Spectrometer calibration stir duration	5 s		
Spectrometer calibration stir speed	30%		
Spectrometer calibration wash pump volume	20.0 mL		
Spectrometer calibration wash stir duration	5 s		
Spectrometer calibration wash stir speed	30%		
Overhead dispense height	10000		

Refinement Settings

Setting	Value	Default value
Turbidity detection method	Spectrometer	Spectrometer
Turbidity wavelength to assess	500.0 nm	500.0 nm
Turbidity maximum absorbance	0.100	0.100
Turbidity probe threshold	50.00	50.00
Exclude turbid points	Yes	Yes
Low intensity warning threshold	100	100
Minimum absorbance change threshold	0.100	0.100
Eigenvector autocorrelation threshold	0.80	0.80



Assay Settings

Sample name:	Pyridoxine HCl	Experiment start time:	5/22/2018 12:19:28 PM
Assay name:	UV-metric psKa	Analyst:	Dorothy Levorse
Assay ID:	18E-22011	Instrument ID:	T311053
Filename:	C:\Sirius_T3\18E-22011_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r		

Refinement Settings (continued)

Setting	Value	Default value
Maximum RMSD severe warning	0.250	0.250
Maximum RMSD warning	0.050	0.050

Tray Information

Title	
Location	B1